Sequence Analysis of the Haemagglutinin of A/Taiwan/1/86, a New Variant of Human Influenza A(H1N1) Virus

By JAMES S. ROBERTSON
National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, U.K.

(Accepted 22 December 1986)

SUMMARY

A/Taiwan/1/86 is representative of newly emerged antigenic variants of influenza A(H1N1) viruses which are readily distinguishable from all previous A(H1N1) isolates. Nucleotide sequence analysis of the haemagglutinin HA1 coding region of A/Taiwan/1/86 suggests that this virus has evolved from viruses circulating in the Hong Kong region in 1982 to 1983. The considerable alteration in antigenicity of this new isolate is likely to have arisen from five amino acid substitutions in a nine amino acid stretch, situated on the outer surface of a short α-helix on the tip of the HA molecule in antigenic site Sb.

Influenza remains a serious disease of epidemic proportions primarily because of the continuous and extensive antigenic variation of the surface haemagglutinin (HA). Antigenic drift of human influenza viruses makes it necessary to assess annually the suitability of the strains used in the preparation of influenza vaccine. Early each year the WHO make a recommendation on the antigenic composition of influenza vaccine based on influenza activity during the preceding months. Influenza activity from October 1985 to February 1986 indicated the need to introduce new A(H3N2) and B antigens, and the infrequent isolation of A(H1N1) viruses and the close antigenic similarity of these few isolates to the reference stain A/Chile/1/83 indicated the continued inclusion of an A/Chile/83-like antigen in vaccine for the 1986/87 season (WHO, 1986a).

However since April 1986 influenza A(H1N1) viruses have been isolated in the Far East which are antigenically readily distinguishable from A/Chile/83 and all current A(H1N1) reference viruses (WHO, 1986b). These isolates show reduced or no haemagglutinin inhibition reactivity with ferret antisera raised against previous A(H1N1) reference strains whilst antiserum against the new prototype A/Singapore/6/86 fails to react with all earlier reference viruses. Furthermore, antibodies in human sera to A/Singapore/86 are detected infrequently and at lower levels compared to A/Chile/83, while vaccines containing A/Chile/83 do not reliably induce antibodies against A/Singapore/86 to adequate levels (WHO, 1986b). Consequently in August 1986, despite the proximity to the time of vaccine administration in the Northern Hemisphere, the WHO recommended that vaccines include an A/Singapore/86-like antigen (WHO, 1986b).

In this report I present the amino acid sequence of the HA1 region of the HA of A/Taiwan/1/86 deduced from the nucleotide sequence of the HA gene (Fig. 1). A/Taiwan/86 is an A/Singapore/86-like virus (WHO, 1986b) which has been used recently to prepare vaccine in the U.S.A. and in several European countries. Comparison of the A/Taiwan/86 HA1 sequence with that derived from other A(H1N1) viruses isolated in previous years (Daniels et al., 1985; Raymond et al., 1986) shows that A/Taiwan/86 has greater similarities to viruses isolated in the Hong Kong region in 1982 to 1983 than to A/Chile/83 or any other H1N1 reference strain. Using H3 numbering (Raymond et al., 1986), amino acid substitutions at residues 5 and 6 which are
Fig. 1. The deduced amino acid sequence of the HA1 region of the HA of A/Taiwan/1/86. Virus was grown in the allantoic cavity of embryonated hens' eggs and the nucleotide sequence of the HA 1 coding region determined by the dideoxy chain-termination technique using synthetic oligonucleotide primers and reverse transcriptase as described (Caton et al., 1982). Differences between A/Taiwan/1/86 and A/Chile/1/83, A/Hong Kong/32/83 (Raymond et al., 1986) and A/Hong Kong/2/82 (Daniels et al., 1985) are indicated. Potential glycosylation sites are underlined. The amino acids are numbered to correspond to the H3 subtype HA following alignment of the cysteine residues as described (Winter et al., 1981).

unique to strains isolated in Hong Kong in these years (A/Hong Kong/2/82 and A/Hong Kong/32/83) (Fig. 1) are also found in A/Taiwan/86; at residues 46 and 53 amino acid substitutions which occur in most 1983 H1N1 isolates including A/Chile/83 were not found in the Hong Kong isolates nor in A/Taiwan/86 (Fig. 1). Additionally, most of the unique nucleotide changes in A/Hong Kong/83 were present in A/Taiwan/86 whereas very few of the unique nucleotide changes found in other 1983 isolates were present (data not shown). These observations suggest that A/Taiwan/86 has evolved from viruses resembling the Hong Kong isolates of 1982 and 1983 rather than from any other characterized strain.

The region of the gene encoding HA1 of A/Taiwan/86 differed from that of A/Chile/83 by 31 base substitutions, resulting in 14 amino acid changes (Fig. 1). Some or all of these substitutions in A/Taiwan/86 compared with A/Chile/83 must be responsible for the considerable antigenic difference between these viruses. The substitutions are indicated in the established three-dimensional structure of an H3 subtype HA in Fig. 2 (Wilson et al., 1981). The substitutions at residues 5, 6, and 46 are located in the stem region and are unlikely to contribute to antigenicity. Residue 53 (K→R) may not be involved in antigenicity due to masking of this region by carbohydrate (Caton et al., 1982). At residue 63 a potential glycosylation site has been created in A/Taiwan/86 by a K→N substitution. Extra carbohydrate in this region may additionally mask a potentially antigenic region in previous isolates. The two substitutions involving residues 129 and 131 have conserved a glycosylation site in the loop forming part of antigenic site Sa (Fig. 1 and 2) (Caton et al., 1982).

Strikingly, five substitutions are located on the external surface of a short α-helix between residues 189 and 197 at the tip of the HA molecule (Fig. 1 and 2). This region is antigenically significant and comprises part of antigenic site Sb (Caton et al., 1982). Since the reappearance of A(H1N1) viruses in 1977 this region has not undergone any significant antigenic drift (Daniels et al., 1985; Raymond et al., 1986). Most mutations found in this region in previous isolates occur at residues 189 and 190 and are not maintained in the evolution of the H1N1 viruses (Daniels et al., 1985; Raymond et al., 1986). These mutations are presumably associated with laboratory
passage of isolates in embryonated hens' eggs. All egg-adapted A(H1N1) isolates contain at least one substitution in HA1 associated with propagation in eggs (Robertson et al., 1986; J. S. Robertson, unpublished results). Characteristic substitutions associated with egg adaptation are E→K at 189, D→N at 190 and D→G or D→N at 225. Thus, N(225) in A/Chile/83, G(225) in A/Hong Kong/82 and K(189) in A/Hong Kong/83 (Fig. 1) probably arose through laboratory passage of these viruses in eggs. Additionally since we have observed E→G substitutions at residue 189 in monoclonal antibody-selected antigenic variants but not E→K (J. S. Robertson, unpublished results), it is likely that the E→G (189) substitution in A/Taiwan/86 is due to immune selection whilst G(225) arose through laboratory passage in eggs. At least four of the five substitutions in the 189 to 197 region are antigenically significant. Substitution at residue 225 has little effect on the antigenicity of the virus as determined by haemagglutination inhibition analyses of A(H1N1) variants containing a single substitution at this residue (J. S.
Robertson, unpublished results). Residue 240, located in antigenic site Ca, has a substitution G→E which may have a role in the altered antigenicity of A/Taiwan/86.

The data indicate that A/Taiwan/86 has evolved from viruses resembling those isolated in Hong Kong in 1982 to 1983. It is reasonable to conclude that the five amino acid substitutions between residues 189 and 197 at the top surface of the HA in a region previously unexposed to antigenic drift have contributed most to the considerable antigenic difference between A/Taiwan/86 and all previous A(H1N1) reference strains.

I thank John Wood and Bob Newman for supplying A/Taiwan/1/86 virus.

REFERENCES


(Received 26 November 1986)